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(7*R*,8*S*)-*cis*-7,8-EPOXY-2-METHYLOCTADEC-17-ENE: A NOVEL TRACE COMPONENT FROM THE SEX PHEROMONE GLAND OF GYPSY MOTH, *Lymantria dispar*

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Abstract—Considering the vast Eurasian distribution of gypsy moth, *Lymantria dispar* (Lepidoptera: Lymantriidae), the many subspecies, and their presence in different lymantriid communities, we tested the hypothesis that *L. dispar* populations in eastern Asia employ one or more pheromone components in addition to the previously known single component pheromone (7*R*,8*S*)-*cis*-7,8-epoxy-2-methyloctadecane [= (+)-disparlure]. Coupled gas chromatographic–electroantennographic detection (GC–EAD) analyses of pheromone gland extracts of female *L. dispar sensu lato* (including both AGM and NAGM) on four GC columns (DB-5, DB-23, DB-210, and SP-1000) revealed a new trace component that eluted just before (DB-5; DB-210) or after (DB-23, SP-1000) disparlure, and elicited strong antennal responses. Isolation of this compound by high-performance liquid chromatography and hydrogenation produced disparlure, suggesting that the new component had the molecular skeleton of disparlure, with one or more double bonds. Of all possible monounsaturated *cis*-7,8-epoxy-2-methyloctadecenes, only *cis*-7,8-epoxy-2-methyloctadec-17-ene co-chromatographed with the insect-produced compound on all GC columns and elicited comparable antennal responses. In field experiments in Honshu (Japan)

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METHODS AND MATERIALS

Experimental Insects

Female pupae of the NAGM were obtained from the laboratory colony maintained at USDA, Beneficial Insects Introduction Research Laboratory, Newark, DE. Additional NAGMs were field collected as late instar larvae at Welch Point, Elkton, Cecil Co., MD (N 39° 32′, W 75° 52′) and held in mesh cages in an outdoor insectary until eclosion of adult moths.

Egg masses of AGM were obtained from several locations in Asia, including Japan (Honshu, Okinawa), Korea (near Seoul and Cheju Island), and China (Hebei and Liaoning Provinces). The larvae were reared on standard gypsy moth diet (Bell et al., 1981) in the USDA Quarantine Facility, Newark, DE. Female pupae were held under a photoperiod of 14L:10D, 24–26°C, and 60–80% relative humidity, whereas male pupae were held at 15°C to retard development. Abdominal tips with pheromone glands of calling, 1- to 3-d-old virgin female moths were removed and extracted for 15–30 min in HPLC-grade hexane. Ampoules with the supernatant of pheromone extracts and male pupae were sent by courier to Simon Fraser University (SFU). Male pupae were held in SFU's Global Forest Quarantine Facility.

Analyses of Pheromone Extract

Aliquots of 1–3 female equivalents (FE) of combined pheromone gland extracts were analyzed by coupled gas chromatographic–electroantennographic detection (GC–EAD) (Arn et al., 1975) with procedures and equipment recently described in detail (Gries et al., 2002). Pheromone extract was fractionated by high-performance liquid chromatography (HPLC), employing a Waters LC 625 high-performance liquid chromatograph equipped with a Waters 486 variable wavelength UV–visible detector set at 210 nm , HP Chemstation software (Rev.A.07.01), and a reverse phase Nova Pak® C_{18} (3.9 mm × 300 mm) column (Waters) eluted with acetonitrile (1 ml/min). For HPLC fractionation, 200 FE of pheromone gland extracts were evaporated to dryness, 50 μl of acetonitrile were added, and the 50-μl extract was injected into the HPLC. Seventy-five 20-sec (200 μl) fractions of this pheromone extract (200 FE) were collected and analyzed individually without concentration by GC–EAD on a DB-5 column. Coupled GC–mass spectrometric (MS) analyses of pheromone extract and of synthetic standards employed a Varian Saturn 2000 ion trap GC–MS fitted with a DB-5 column (30 m × 0.25 mm ID; J&W Scientific, Folsom, CA).

General Methods and Instrumentation

Tetrahydrofuran (THF) and *N*-methylpyrrolidinone (NMP) were dried by standard methods. Oven-dried glassware was assembled hot under Ar flow, and

maintained under Ar; liquids were transferred by cannula under Ar pressure. Dry LiCl and $CuCl_2$ were weighed out in a dry box. Infrared spectra were recorded on Perkin-Elmer Paragon 1000 and 1600 FT–IR instruments. Optical rotations were measured on Jasco DIP-1000 and Perkin-Elmer 341 polarimeters. Nuclear magnetic resonance (NMR) spectroscopy of synthetic compounds was conducted on Bruker 300 (at 300 MHz for ^{1}H and 75 MHz for ^{13}C) and Varian AS500 (at 499.77 MHz for ^{1}H and 125.68 MHz for ^{13}C) spectrometers, with chemical shifts reported in ppm relative to TMS (^{1}H, δ 0.00) and $CDCl_3$ (^{13}C, δ 77.00). Elemental analyses were performed using a Carlo-Erba model 1106 elemental analyzer.

Syntheses

(*2′S,3′R*)-*8′-Methyl-cis-2′, 3′-Epoxy-1-Nonyl (1S)-10-Camphorsulfonate* (***2***) (Figure 1). (2′*S*, 3′*S*)-4′-Bromo-*cis*-2′,3′-epoxy-1-butyl (1*S*)-10-camphorsulfonate (**1**, >99% ee; Aldrich) (11.0 g, 28.9 mmol) was melted *in vacuo*, and dissolved in THF (30 ml). The solution was cannulated into a 300-ml 3-neck flask fitted with a low-temperature thermometer, magnetic stir bar, and a pressure-equalizing 125-ml

3.66 (d, J = 15.1 Hz, 1H), 3.26 (m, 1H), 3.06 (m, 1H), 3.06 (d, J = 15.1 Hz, 1H), 2.51–2.35 (m, 2H), 2.14 (t, J = 4.5 Hz, 1H), 1.96 (d, J = 18.5 Hz, 1H), 1.12 (s, 3H), 0.89 (s, 3H), 0.87 (d, J = 6.7 Hz, 6H); ^{13}C NMR δ 214.27, 68.60, 57.82, 56.68, 53.32, 48.00, 47.09, 42.64, 42.42, 38.70, 27.89, 27.78, 27.03, 26.79(2), 24.76, 22.52(2), 19.65, 19.58. HRMS (ESI) calcd for $C_{20}H_{34}O_5SNa$ (M^+ + Na) 409.2019, found 409.2004.

*(2R,3R)-1-Iodo-8-Methyl-cis-2,3-Epoxynonane (**3**).* To a solution of **2** (7.00 g, 18.1 mmol) in reagent grade acetone (50 ml) in an Ar-swept flask were added a few mg of $NaHCO_3$, and a solution of NaI (5.5 g, 37 mmol) in acetone (80 ml). Precipitation occurred almost at once. The magnetically stirred mixture was heated in a 45° bath (reflux condenser, positive Ar pressure) for 41 hr (no remaining **2** by NMR analysis). The solid was collected by suction filtration, and washed thoroughly with reagent acetone; the yellow filtrate was concentrated at 40°–45°, and taken up in H_2O (100 ml) and 10% Et_2O–hexanes (150 ml). The organic phase was washed with dilute Na_2SO_3 and water, dried (Na_2SO_4–$MgSO_4$), filtered through silica gel (5 g), stripped of solvent, and pumped under vacuum to leave 4.71 g (92%) of colorless oil: $[\alpha]_D^{26}$ +73.1° (*c* 4.85, $CHCl_3$); IR (film) 2952, 1466, 1166 cm^{-1}; ^{1}H NMR δ 3.36–3.27 (m, 2H, H-1 and H-2), 3.08–3.05 (m, 1H, H-3), 3.05–2.96 (m, 1H, H-1), 1.62–1.16 (3 m's, 9H), 0.88 (d, J = 6.6 Hz, 6H); ^{13}C NMR δ 59.91, 56.74, 38.74, 27.83, 27.21, 27.05, 26.77, 22.57(2), 1.33. HRMS (ESI) calcd for $C_{10}H_{19}IONa$ (M^+ + Na) 305.0373, found 305.0387.

*(7R,8S)-cis-7,8-Epoxy-2-Methyloctadec-17-ene (**4**).* A solution of 1-nonen-9-yl-magnesium bromide [freshly prepared from 2.70 g (13.20 mmol) of 9-bromo-1-nonene with 2 equiv. of magnesium turnings] in 50 ml of THF was added dropwise into a mixture of iodoepoxide **3** (1.24 g, 4.40 mmol), CuI (0.167 g, 0.88 mmol), distilled HMPA (4.60 ml, 26.40 mmol), and dry THF (2 ml) at −23°C. After stirring for 25 min, the reaction mixture was quenched with aq. NH_4Cl solution. The products were extracted with hexane–ether (1:1), the extracts were washed (water, saturated aq. $Na_2S_2O_3$, brine), and dried (anhyd. $MgSO_4$), and solvents and low-boiling byproducts were removed *in vacuo*. Flash chromatography (50 g SiO_2, 0–4% ether in hexane) yielded 0.813 g (2.90 mmol) of pure **4** (66%). $[\alpha]_D^{21}$ +2.3° (*c* 0.48, $CHCl_3$); IR (film) 2930, 2856, 1641, 1466, 1385, 1366, 992, 909 cm^{-1}; ^{1}H NMR δ 5.81 (m, 1H), 4.99 (dd, J =17.1, 2.1 Hz, 1H), 4.93 (d, J = 10.3 Hz, 1H), 2.90 (m, 2H), 2.04 (td, J = 7.6, 6.8 Hz, 2H), 1.15–1.57 (4 m's, 23 H), 0.87 (d, J = 6.6 Hz, 6H); ^{13}C NMR δ 139.18, 114.10, 57.24, 38.88, 33.78, 29.59, 29.49, 29.36, 29.08, 28.91, 28.90, 27.88, 27.85, 27.80, 27.32, 26.84, 26.58, 22.61, 22.60; Anal. calcd. for $C_{19}H_{36}O$ (%): C, 81.36; H, 12.94, found: C, 80.96, H, 12.91.

*(2′R,3′S)-8′-Methyl-cis-2′,3′-Epoxy-1-Nonyl (1S)-10-Camphorsulfonate (**2′**).* This epoxide was prepared from (2′*R*,3′*R*)-4′-bromo-*cis*-2′,3′-epoxy-1-butyl (1*S*)-10-camphorsulfonate (**1′**, >99% ee; Aldrich) (11.0 g, 28.9 mmol) essentially as described for synthesis of **2**, except that the amounts of Li_2CuCl_4 and NMP were

doubled, and sufficient 4-methylpentylmagnesium chloride was added to consume all of **1′**. No ring-opened alcohol was detected. A single column chromatography of the crude product (silica gel, elution as for **2**) gave 6.10 g of ∼98% pure **2′** (54%). Purified **2′**: $[\alpha]_D^{26}$ +40.6° (*c* 4.65, $CHCl_3$); IR (film) 2954, 1748, 1365, 1172, 969 cm^{-1}; ^1H NMR δ 4.50–4.44 (dd, $J = 11.4, 4.2$ Hz, 1H), 4.34–4.28 (dd, $J = 11.7$, 7.1 Hz, 1H), 3.66 (d, $J = 15.1$ Hz, 1H), 3.27 (m, 1H), 3.49–3.42 (m, 2H), 3.11 (d, $J = 15.1$ Hz, 1H), 3.08 (m, 1H), 2.50–2.35 (m, 2H), 2.14 (t, $J = 4.3$ Hz, 1H), 2.12–2.03 (m, 1H), 1.96 (d, $J = 18.5$ Hz, 1H), 1.12 (s, 3H), 0.89 (s, 3H), 0.87 (d, $J = 6.6$ Hz, 6H); ^{13}C NMR δ 214.21, 68.46, 57.86, 56.69, 53.34, 47.95, 47.18, 42.64, 42.41, 38.69, 27.86, 27.77, 27.02, 26.79(2), 24.85, 22.52(2), 19.66, 19.59. HRMS (ESI) calcd, for $C_{20}H_{34}O_5SNa$ (M^++ Na) 409.2019, found 409.1999.

*(2S,3S)-1-Iodo-8-Methyl-cis-2,3-Epoxynonane (**3′**).* This iodomethyl epoxide, prepared from **2′** in 93% yield as described for enantiomer **3**, was obtained as a colorless oil: $[\alpha]_D^{25}$ –73.4° (*c* 4.80, $CHCl_3$); all other physical data were identical to those recorded for **3**.

*(7S,8R)-cis-7,8-Epoxy-2-Methyloctadec-17-ene (**4′**).* Compound **4′** was prepared from iodoepoxide 3′ under the same conditions as described for **4** with 58% yield. $[\alpha]_D^{21}$ –2.1° (*c* 0.71, $CHCl_3$); all spectral data were identical to those recorded for **4**.

Field Experiments

All field experiments employed a complete randomized block design with 10 blocks (replicates) each, and were conducted in forests (N 39° 52′, E 141° 23′ and N 39° 53′, E 141° 18′) near (<35 km) the city of Morioka (Iwate Prefecture, Japan). *Lymantria dispar* experiments 1–4 were set up in forests with mixed oak, birch, and maple, and the *L. monacha* experiment (Exp. 5) in a forest with Japanese larch, *Larix leptolepsis*. Delta-like traps were made from 2-l milk cartons (Gray et al., 1984), coated with Tanglefoot (The Tanglefoot Company, Grand Rapids, Michigan, PA), and suspended from trees 1.5 m above ground at 15 to 20-m spacing. They were baited with a gray sleeve stopper (West Pharmaceutical Services, Lionville, PA) impregnated with candidate pheromone components in HPLC-grade hexane.

Experiment 1 tested 7*R*8*S*-epo-2me-17-ene-18Hy (50 μg) and 7*S*8*R*-epo-2me-17-ene-18Hy (50 μg) singly and in combination. Experiment 2 compared the relative attractiveness of 7*R*8*S*-epo-2me-17ene-18Hy (50 μg) with that of (+)-disparlure (50 μg). Experiment 3 tested whether attractiveness of (+)-disparlure could be enhanced by addition of 7*R*8*S*-epo-2me-17ene-18Hy (0.5, 5, or 50 μg). Experiment 4 explored whether attractiveness of (+)-disparlure was affected by addition of either 7*R*8*S*-epo-2me-17ene-18Hy (0.5 μg), 7*S*8*R*-epo-2me-17ene-18Hy (0.5 μg), or both (0.5 μg each). Final experiment 5 investigated whether 7*R*8*S*-epo-2me-17ene-18Hy enhanced species specificity of the *L. dispar* pheromone by testing the *L. monacha* pheromone blend

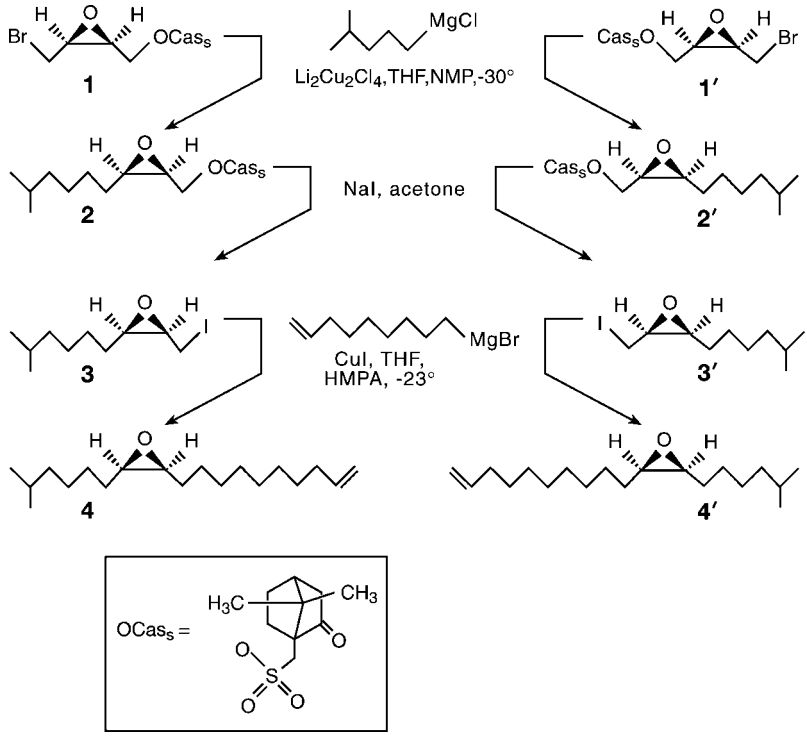

FIG. 1. Scheme for enantioselective syntheses of (7*R*,8*S*)-*cis*-7,8-epoxy-2-methyloctadec-17-ene (**4**), and (7*S*,8*R*)-*cis*-7,8-epoxy-octadec-17-ene (**4′**).

[(7*R*,8*S*)-*cis*-7,8-epoxy-octadecane = (+)-monachalure (50 μg); (+)-disparlure (50 μg); (*Z*)-2-methyl-7-octadecene (2me-*Z*7-18Hy) (5 μg)] vs. the *L. monacha* pheromone blend in combination with 7*R*8*S*-epo-2me-17-ene-18Hy.

Trap catch data were subjected to nonparametric analyses of variance (Friedman's test) followed by comparison of means by Scheffé test (Zar, 1984; SAS/STAT, 1988). In all analyses, $\alpha = 0.05$.

RESULTS AND DISCUSSION

In GC–EAD analyses of pheromone gland extracts of female *L. dispar* from various geographic locations, a previously unknown component elicited strong antennal responses (Figure 2). Even in concentrated pheromone gland extracts [>300 FE], the unknown occurred below detection threshold of the GC–MS, and needed to be identified without spectroscopic data.

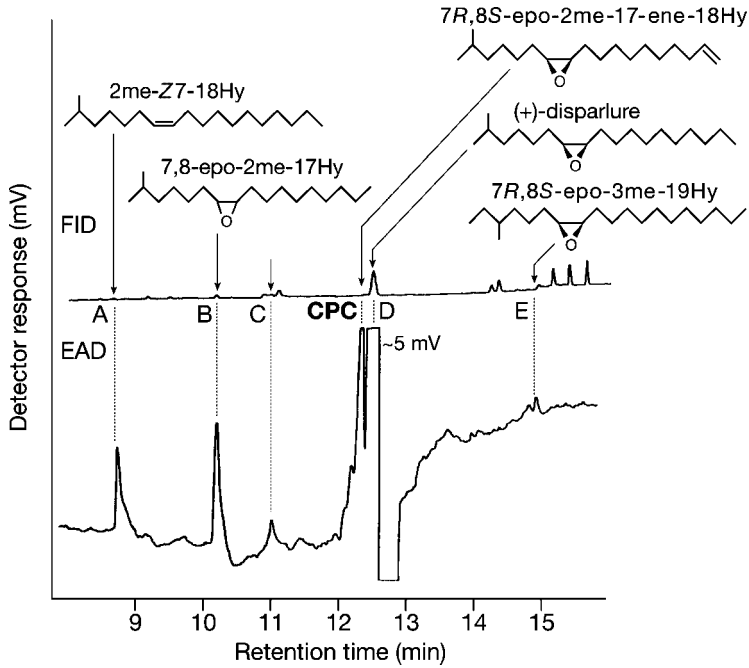

FIG. 2. Flame ionization detector (FID) and electroantennographic detector (EAD: male *L. dispar* antenna) responses to 1 FE of *L. dispar* pheromone gland extract. Chromatography: DB-5 column; 100°C (1 min), 20°C per min to 190°C (held for 8 min), then 25°C per min to 280°C. Except for (+)-disparlure, all components eliciting antennal responses occurred below detection threshold of the FID. Compound identities: **A** = (*Z*)-2-methyl-7-octadecene; **B** = *cis*-7,8-epoxy-2-methylheptadecane (absolute configuration not yet determined); **C** = unknown; **CPC** = candidate pheromone component = (7*R*,8*S*)-*cis*-7,8-epoxy-2-methyloctadec-17-ene; **D** = (+)-disparlure = (7*R*,8*S*)-*cis*-7,8-epoxy-2-methyloctadecane; **E** = (7*R*,8*S*)-*cis*-7,8-epoxy-3-methylnonadecane.

The GC–EAD analyses of pheromone gland extracts on four GC columns (DB-5, DB-23, DB-210, SP-1000) indicated that the unknown component had retention indices (RI) (Van den Dool and Kratz, 1963) of 2027 (DB-5), 2403 (DB-23), 2297 (DB-210), and 2304 (SP-1000) relative to straight-chain alkanes. Retention-indices assignments for the component on both DB-23 and SP-1000 columns remained tentative, because the compound eluted just after disparlure, and elicited only weak antennal responses, possibly due to an antennal refractory phase after a strong response to disparlure.

To confirm correct assignment of RIs for the compound on DB-23 and SP-1000 columns, seventy-five 20-sec (200 μl) HPLC fractions of pheromone extract (200 FE) were analyzed by GC–EAD on a DB-5 column. The fraction containing

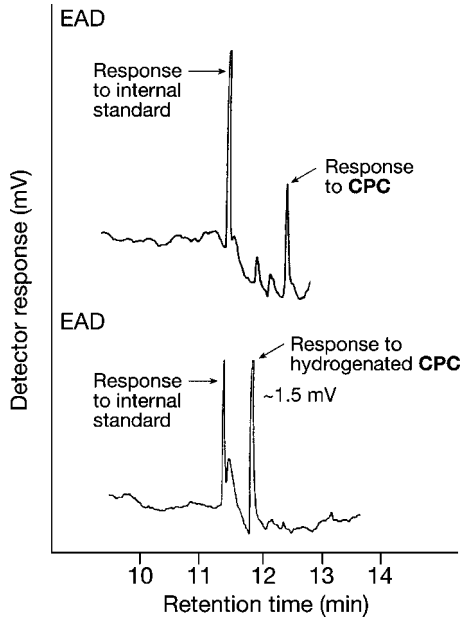

FIG. 3. Electroantennographic detector (EAD: male *Lymantia dispar* antenna) responses to the HPLC-isolated CPC before (top) and after (bottom) hydrogenation. Chromatography: DB-23 column; 100°C (1 min), 10°C per min to 200°C. Corresponding FID traces are omitted. Stronger response to hydrogenated CPC (bottom) may be explained by a more concentrated (×10) sample. (7*R*,8*S*)-*cis*-7,8-Epoxyoctadecane [(+)-monachalure (10 pg)] was used as an internal standard.

the unknown compound was further analyzed on all four columns, revealing antennal responses with the familiar RIs of 2027 (DB-5), 2403 (DB-23), 2297 (DB-210), and 2304 (SP-1000) (as mentioned earlier). Calculations of intercolumn RI differences (e.g., RI on DB-5 minus RI on DB-23; RI on SB-1000 minus RI on DB-23) suggested that the compound might be a monounsaturated epoxide. Hydrogenation and GC–EAD analyses of the fraction (Figure 3) exhibited a different antennal response with RIs on all columns consistent with those of disparlure. To confirm that the unknown compound had the molecular skeleton of disparlure, related 5,6-, 6,7-, 8,9-, and 9,10-epoxy-2-methyloctadecanes were synthesized (Table 1). Their GC characteristics were similar or identical to those of disparlure (Table 1), but their EAD activities were significantly lower.

To determine the double bond position in the unknown (= unsaturated disparlure), all possible *cis*-7,8-epoxy-2-methyloctadecenes were synthesized (Table 2). Of these, only *cis*-7,8-epoxy-2-methyloctadec-17-ene had GC- and EAD-characteristics entirely consistent with those of the novel compound.

TABLE 1. RETENTION INDICES RELATIVE TO STRAIGHT-CHAIN ALKANES OF VARIOUS SATURATED EPOXIDES SYNTHESIZED FOR THE IDENTIFICATION OF THE CANDIDATE PHEROMONE COMPONENT (CPC) IN FIGURE 2

	Type of column		
	DB-23	DB-5	DB-210
Synthetic chemical	*trans*/*cis*	*trans*/*cis*	*trans*/*cis*
5,6-Epoxy-2-methyloctadecane	2321/2343	2018/2030	2289/2309
6,7-Epoxy-2-methyloctadecane	2316/2340	2015/2030	2286/2307
7,8-Epoxy-2-methyloctadecane[a]	2315/2337	2015/2028	2287/2305
8,9-Epoxy-2-methyloctadecane	2315/2337	2015/2028	2287/2306
9,10-Epoxy-2-methyloctadecane	2315/2337	2015/2028	2287/2306

[a] Disparlure.

TABLE 2. RETENTION INDICES RELATIVE TO STRAIGHT-CHAIN ALKANES AND COMPARATIVE ANTENNAL RESPONSES ELICITED BY *cis*-7,8-EPOXY-2-METHYLOCTADECENES

	Retention indices			
Compound	DB-23	DB-5	DB-210	EAD-activity*
cis-7,8-Epoxy-2-methyl-Δ**1**-*ENE*-18Hy	2442	2050	2343	+
cis-7,8-Epoxy-2-methyl-Δ**2**-*ENE*-18Hy	2425	2056	2329	
cis-7,8-epoxy-2-methyl-***Z*3**-*ENE*-18Hy	2334	2009	2267	+
cis-7,8-epoxy-2-methyl-***Z*4**-*ENE*-18Hy	2374	2019	2300	+
cis-7,8-epoxy-2-methyl-***Z*10**-*ENE*-18Hy	2354	2004	2284	
cis-7,8-epoxy-2-methyl-***Z*11**-*ENE*-18Hy	2356	2006	2281	
cis-7,8-epoxy-2-methyl-***Z*12**-*ENE*- 18Hy	2363	2005	2286	+
cis-7,8-epoxy-2-methyl-***Z*13**-*ENE*-18Hy	2380	2014	2301	
cis-7,8-epoxy-2-methyl-***Z*14**-*ENE*-18Hy	2390	2020	2308	
cis-7,8-epoxy-2-methyl-***Z*15**-*ENE*-18Hy	2396	2025	2315	+
cis-7,8-epoxy-2-methyl-***Z*16**-*ENE*-18Hy	2431	2045	2338	
cis-7,8-epoxy-2-methyl-Δ**17**-*ENE*-18Hy	2402	2020	2315	++
cis-7,8-epoxy-2-methyl-***E*3**-*ENE*-18Hy	2332	2001	2263	
cis-7,8-epoxy-2-methyl-***E*4**-*ENE*-18Hy	2361	2021	2292	
cis-7,8-epoxy-2-methyl-***E*10**-*ENE*-18Hy	2362	2020	2292	
cis-7,8-epoxy-2-methyl-***E*11**-*ENE*-18Hy	2354	2014	2280	
cis-7,8-epoxy-2-methyl-***E*12**-*ENE*-18Hy	2354	2010	2285	
cis-7,8-epoxy-2-methyl-***E*13**-*ENE*-18Hy	2366	2017	2288	
cis-7,8-epoxy-2-methyl-***E*14**-*ENE*-18Hy	2364	2016	2289	
cis-7,8-epoxy-2-methyl-***E*15**-*ENE*-18Hy	2372	2020	2290	
cis-7,8-epoxy-2-methyl-***E*16**-*ENE*-18Hy	2392	2030	2307	

"+" indicates strong EAD-activity.
"++" indicates very strong EAD-activity.

Enantiomers of *cis*-7,8-epoxy-2-methyloctadec-17-ene were synthesized according to the schemes in Figure 1. In the conversion of **1** to **2**, only 1 mol% of the copper complex catalyst and a minimal amount of NMP was used. Possibly as a consequence, the reaction could not be pushed to completion with excess Grignard reagent, which apparently reacted preferentially with already formed **2** to open the

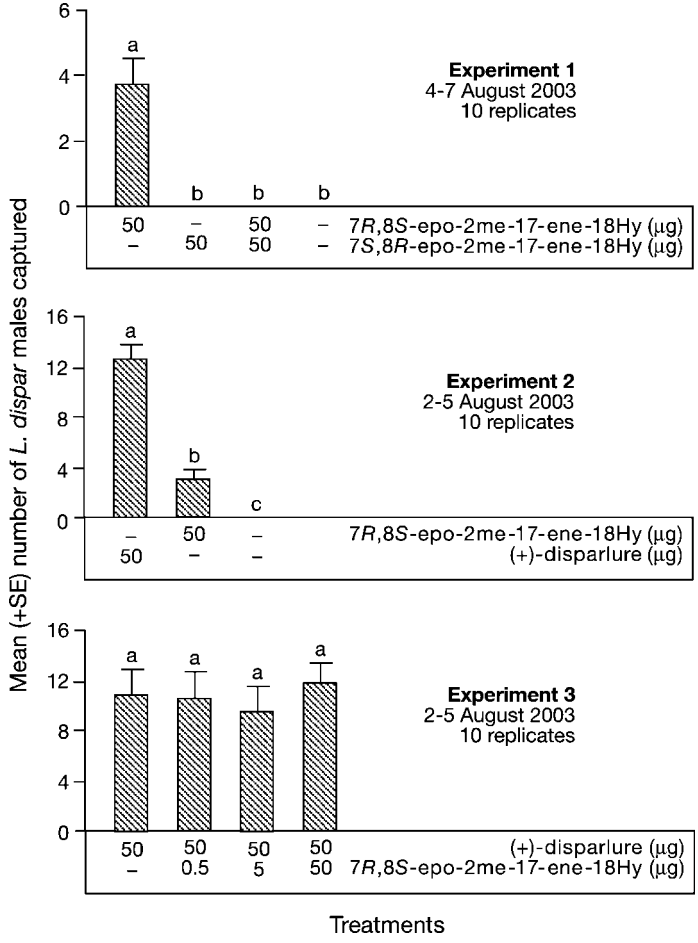

FIG. 4. Captures of male gypsy moths, *Lymantria dispar,* in experiments 1–3 in sticky traps baited with candidate pheromone components [(+)-disparlure = (7*R*,8*S*)-*cis*-7,8-epoxy-2-methyloctadecane; 7*R*8*S*-epo-2me-17-ene-18Hy = (7*R*,8*S*)-*cis*-7,8-epoxy-2-methyloctadec-17-ene; 7*S*,8*R*-epo-2me-17-ene-18Hy = (7*S*,8*R*)-*cis*-7,8-epoxy-2-methyloctadec-17-ene]; mixed forests near Morioka (Honshu, Japan); in each experiment, bars with different letter superscripts are significantly different, $\alpha = 0.05$.

epoxide ring at the bond distal to the camphorsulfonate. The ring-opened alcohol thus formed could not be separated from **2** by chromatography. However, treatment of the mixture with K_2CO_3 in methanol rapidly and selectively converted the alcohol to the terminal epoxide, which then was easily separated by chromatography. In the conversion of **1**′ to **2**′, with the relative amounts of catalyst and NMP doubled, coupling went to completion, and no ring-opened alcohol was detected.

The biological role of (7*R*,8*S*)-*cis*-7,8-epoxy-2-methyloctadec-17-ene (7*R*8*S*-epo-2me-17-ene-18Hy) is not yet clear. It is present in pheromone glands (Figure 2), but it is not known whether it is released by calling females. By itself it attracted male moths (Figure 4; Exps. 1, 2). However, it neither enhanced attractiveness of (+)-disparlure (Figure 4, Exp. 3; Figure 5, Exp. 4) nor did it affect

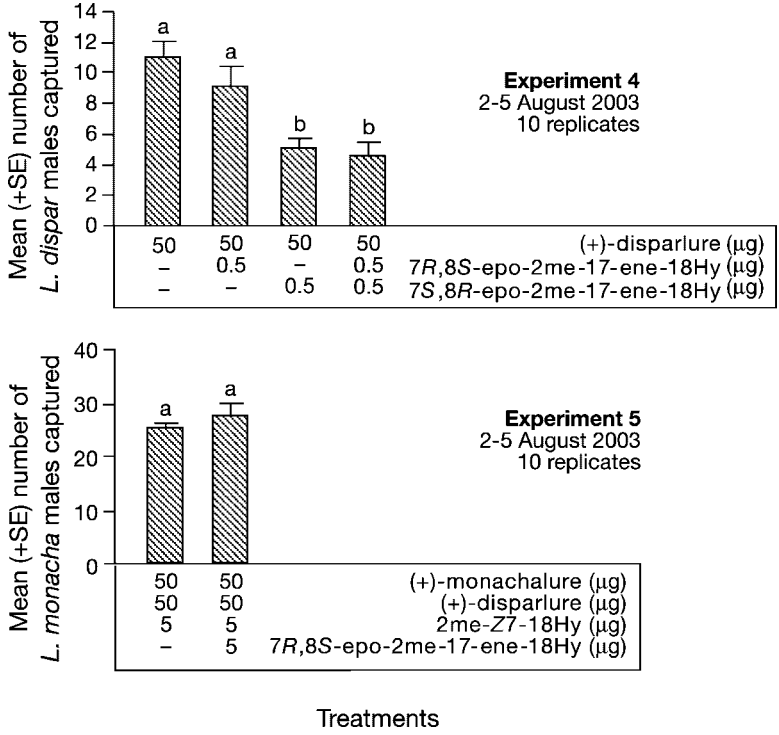

FIG. 5. Captures of male gypsy moths, *Lymantria dispar* (Exp. 4), and male nun moths, *Lymantria monacha* (Exp. 5), in sticky traps baited with candidate pheromonal and allomonal components [(+)-monachalure = (7*R*,8*S*)-*cis*-7,8-epoxy-octadecane; 2me-*Z*7-18Hy = (*Z*)-2-methyl-7-octadecene; other abbreviations as in caption of Figure 4]; Larch (*Larix* spp.) forest near Morioka (Honshu, Japan); in each experiment, bars with different letter superscripts are significantly different, $\alpha = 0.05$.

the specificity of the blend with regard to *L. monacha* (Figure 5; Exp. 5). A discernible effect of 7*R*8*S*-epo-2me-17-ene-18Hy may yet be discovered, if it were tested in a context other than long-range attraction of males (Cameron, 1981), or if it were dispensed from a more suitable release device. In field experiments with codling moths, *Cydia pomonella*, for example, secondary pheromone components failed to affect blend attractiveness when they were dispensed from rubber septa (El-Sayed et al., 1999a), but enhanced blend attractiveness when they were dispensed from a piezoelectric sprayer (El-Sayed et al., 1999b; El-Sayed and Trimble, 2002). It is also possible (although not likely) that the effects of 7*R*8*S*-epo-2me-17-ene-18Hy will only be manifested when this component is tested together with other components found in pheromone gland extracts, such as *cis*-7,8-epoxy-2-methylheptadecane or (7*R*,8*S*)-*cis*-7,8-epoxy-3-methylnonadecane (Figure 2; Gries et al., 1996; unpublished). Alternatively, 7*R*8*S*-epo-2me-17-ene-18Hy may be a trace component that provides insight into pheromone phylogeny, and may serve as a pheromone component or antagonist in *Lymantria* congeners.

Furthermore even though 7*R*8*S*-epo-2me-17-ene-18Hy had no detectable role in the *L. dispar* population of northern Honshu, it may be important in other *L. dispar* populations. In *L. monacha*, for example, (+) -disparlure is the most and least important pheromone component in populations in Bohemia (Europe) and Honshu (Japan), respectively (Gries et al., 2001). This contrasting role of (+)-disparlure may be attributed to reproductive character displacement caused by congeneric *L. fumida* on Honshu (Gries et al., 2001), which utilizes (+)-disparlure as its major pheromone component (Schaefer et al., 1999). Considering the wide distribution of *L. dispar*, and its presence in many different lymantriid communities, there may be some communities in which *L. dispar* maintains specificity of sexual communication by using 7*R*8*S*-epo-2me-17ene-18Hy in addition to, or as a substitute for, (+)-disparlure. Until such communities are found, synthetic (+)-disparlure as a single-component lure remains the best lure for detection of (Asian) *L. dispar.*

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